

WHAT IS CLAIMED IS:

1. A method of establishing a clonal embryonic stem cell line capable of sustaining a phenotype of normal embryonic stem cells following at least eight months of *in vitro* culturing, the method comprising culturing an individual embryonic stem cell for at least eight months in a serum-free medium, thereby establishing the clonal embryonic stem cell line capable of sustaining said phenotype of normal embryonic stem cells following at least eight months of *in vitro* culture.

2. The method of claim 1, wherein said individual embryonic stem cell is a human embryonic stem cell.

3. The method of claim 1, wherein the phenotype of normal embryonic stem cells is characterized by a normal karyotype, a non-increasing population doubling time selected from a range of 28 to 42 hours, a non-decreasing telomere length, non-decreasing telomerase activity and pluripotentiality.

4. The method of claim 3, wherein said non-increasing population doubling time is selected from a range of 33 to 37 hours.

5. The method of claim 3, wherein said non-decreasing telomere length is selected from a range of 4 to 16 kb.

6. The method of claim 3, wherein said non-decreasing telomere length is selected from a range of 8 to 12 kb.

7. The method of claim 3, wherein said pluripotentiality is characterized by the capacity to differentiate into endodermal, mesodermal and ectodermal cells.

8. The method of claim 1, further comprising the step of obtaining said individual embryonic stem cell from a source selected from the group consisting of an embryonic stem cell culture, a blastocyst inner cell mass, a blastocyst, embryonic germ cells, an embryonic germ cell culture, an embryo and a fetus prior to said step of culturing.

9. The method of claim 8, wherein said step of obtaining said individual embryonic stem cell from said blastocyte inner mass is effected by:

- (a) isolating a blastocyst;
- (b) isolating cells from the inner cell mass of said blastocyst;
- (c) culturing said cells from the inner cell mass on mouse embryonic feeder fibroblasts, thereby generating an inner cell mass-derived cell mass;

33

- (d) dissociating said inner cell mass-derived cell mass into dissociated cells;
- (e) culturing said dissociated cells on mouse embryonic feeder fibroblasts, thereby generating dissociated cell-derived colonies;
- (f) selectively harvesting from among said dissociated cell-derived colonies a colony with morphologically compact cells, cells with high nucleus-to-cytoplasm ratio and/or cells with prominent nucleoli; and
- (g) dissociating said colony with morphologically compact cells, cells with high nucleus-to-cytoplasm ratio and/or cells with prominent nucleoli into individual cells thereby obtaining said individual embryonic stem cell.

10. The method of claim 1, wherein said serum-free medium includes feeder fibroblasts.

11. The method of claim 10, wherein said feeder fibroblasts are murine.

12. The method of claim 10, wherein said feeder fibroblasts are embryonic.

34

13. The method of claim 1, wherein said serum-free medium includes 0.4 to 40 ng/ml bFGF.

14. The method of claim 1, wherein said serum-free medium includes 1 to 16 ng/ml bFGF.

15. The method of claim 1, wherein said serum-free medium includes 2 to 8 ng/ml bFGF.

16. The method of claim 1, wherein said serum-free medium includes 4 ng/ml bFGF.

17. A clonal human embryonic stem cell line being capable of sustaining a normal embryonic stem cell phenotype following at least eight months of *in vitro* culturing.

18. The clonal human embryonic stem cell line of claim 17, wherein said *in vitro* culturing is effected on mouse embryonic feeder fibroblasts in serum-free medium supplemented with basic fibroblast growth factor.

19. The clonal human embryonic stem cell line of claim 17, wherein the phenotype of normal embryonic stem cells is characterized by a normal karyotype, a non-increasing population doubling time selected from a range of

28 to 42 hours, a non-decreasing telomere length, non-decreasing telomerase activity and pluripotentiality.

20. The clonal human embryonic stem cell line of claim 17, wherein said non-increasing population doubling time is selected from a range of 33 to 37 hours.

21. The clonal human embryonic stem cell line of claim 17, wherein said non-decreasing telomere length is selected from a range of 4 to 16 kb.

22. The clonal human embryonic stem cell line of claim 17, wherein said non-decreasing telomere length is selected from a range of 8 to 12 kb.

23. The clonal human embryonic stem cell line of claim 17, wherein said pluripotentiality is characterized by the capacity to differentiate into endodermal, mesodermal and ectodermal cells.

24. A clonal human embryonic stem cell line being capable of sustaining a normal embryonic stem cell phenotype following at least twelve months of *in vitro* culturing.